

NEW GENETIC DATA ON *GENISTA ANGLICA* L. VERSUS *GENISTA ANCISTROCARPA* SPACH (FABACEAE, FABALES) IN THE IBERIAN PENINSULA AND MOROCCO. PHYLOGEOGRAPHIC CLUES

Miguel GERALDES¹*, João Paulo FONSECA², Carlos NETO¹ & José Carlos COSTA²

¹ Centro de Estudos Geográficos, Instituto de Geografia e Ordenamento do Território, Universidade de Lisboa, Alameda da Universidade 1600-214, Lisboa, Portugal

² Centro de Biociências. ISPA-IU. R. Jardim do Tabaco, 34, 1149 - 041 Lisboa, Portugal

³ CBAA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349017 Lisboa, Portugal

* Correspondence author: mgeraldes@campus.ul.pt

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ABSTRACT. *New genetic data on Genista anglica L. versus Genista ancistrocarpa Spach (Fabaceae, Fabales) in the Iberian Peninsula and Morocco. Phylogeographic clues.* The genetic analysis through the nuclear ribosomal DNA of some Iberian and NW Morocco populations of *Genista ancistrocarpa* Spach and *G. anglica* L. has validated the separation of these two sister taxa as two distinct genetic entities, strengthening the already described morphological differentiation. The studied populations neatly show the *G. ancistrocarpa* as a coastal taxon with occidental Iberian and NW Morocco distribution, contrary to the *G. anglica*. The distribution areas of the two species appear to be distinct and with an important biogeographical significance. The coastal clade is determined by populations of *G. ancistrocarpa*, and outlines a biogeographic region that seems to depart genetically from the remaining peninsular populations, also in several molecular analyses of other plants and animals by many authors, supporting the idea of a distinct evolution from the Miocene onwards, when the installation of dry and hot conditions has pushed the moisture-dependent populations of *G. ancistrocarpa* toward the westernmost sectors of Iberia and Morocco with an atlantic influence.

Key words. Chorology, *Genista anglica*, *Genista ancistrocarpa*, sublittoral fens, Iberian Peninsula, Morocco.

RESUMO. *Novos dados genéticos sobre a Genista anglica L. versus Genista ancistrocarpa Spach (Fabaceae, Fabales) na Península Ibérica e Marrocos. Indícios filogeográficos.* A análise genética de ADN nuclear ribossomal, levada a cabo em algumas populações peninsulares e do NW de Marrocos de *Genista ancistrocarpa* e de *G. anglica*, permitiu validar estes dois taxa irmãos como entidades genéticas distintas, reforçando, desta forma, a sua diferenciação morfológica. As análises permitem definir a *G. ancistrocarpa* como um taxon litoral com distribuição oeste ibérica e NW de África, por oposição à *G. anglica*. As áreas de distribuição das duas espécies parecem ser distintas e com importante significado biogeográfico. O

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clado litoral definido pelas populações de *G. ancistrocarpa* define um território biogeográfico, que parece destacar-se geneticamente das restantes populações peninsulares, em diversas plantas e animais analisados geneticamente por vários autores, reforçando a ideia de uma evolução distinta a partir do Mioceno médio, quando a instalação de um clima seco e quente empurrou e isolou uma parte da população da higrófila *G. ancistrocarpa* para ocidente, onde podiam receber uma influência atlântica.

Palavras-chave. Corologia, *Genista anglica*, *Genista ancistrocarpa*, turfeiras sublitorais, Península Ibérica, Marrocos.

INTRODUCTION

While the most species of the *Genista* genus thrive on dry soils, the *Genista anglica* L. and *Genista ancistrocarpa* Spach are two species of atlantic kinship, which have their southernmost chorology on wet, acidic, poor, peat-rich soils in Portugal, Spain and Morocco, commonly near drainage lines with low energy like creeks, ponds and lakes, whether very near to the coastline (sublittoral ecosystems) or in the non-arid mountains (Costa *et al.* 1998). These habitats occur where annual mean rainfall reaches over 800 mm up to 2800 mm or, concerning areas more to the south, where the water-table lies near the surface and springs out, creating water-logged conditions.

Recent taxonomic discussion has been performed about the other southern chorology, the isolated populations in Calabria, southern Italy, on two of its mountain ranges - the *Aspromonte* and the *Sila*, formerly considered as *G. anglica*, which have been recently described as a new endemic taxon, *G. silana* Brullo, Gangale & Spamp for *Sila* range and *G. brutia* Brullo, Scelsi & Spamp. (Brullo *et al.* 2001) for the *Aspromonte* range, albeit not yet acknowledged by a great many authors. These will not be considered in this article.

The present work aims to contribute to shed light on the systemic reports between southwestern populations of the two commonly accepted sister taxa of wet-soil *Genista*: *G. anglica* and *G. ancistrocarpa*, exploring the comparison between pheno-taxonomic traits

and new data from nuclear ribosomal DNA for these very closely-related taxa.

It has indeed been accepted that *G. anglica* and *G. ancistrocarpa* are morphologically analogous in many phenotypic traits. In the Iberian Flora (Talavera, 1999), they are separated mainly by the number of leaflets in the armpit of the infertile talus, being unifoliate in *G. anglica*, yet trifoliate in non-flowering branches in *G. ancistrocarpa*.

In the Iberian Peninsula, the *G. ancistrocarpa* occurs mainly in lowlands (0-20 m), near the ocean, with local edaphic fresh-water compensation, originating peaty fen complexes neighboured by hygrophilous heathlands, while *G. anglica* appears at higher elevations (10-1800 m), where greater values of annual precipitation are recorded, normally more than 800 mm (Talavera, 1999).

Study area

In order to clarify the phylogenetic relationships among populations of the two species (*G. ancistrocarpa* and *G. anglica*) of the Southwestern Iberian Peninsula and Northwestern Morocco, we performed some genetic analysis using specimens of *G. anglica* sampled in the following places: Sierra de Cebollera (Northern Iberian System Range), Sierra de los Ancares (Cantabrian System Range), Serra da Estrela (Central Iberian System Range), and Morais Massif (Trás-os-Montes). For the *G. ancistrocarpa*, the leaves were sampled from the Graben of Chaves (Trás-os-Montes), the Serra de Montemuro

(Northwestern Portuguese Mountains), the Sado Estuary (Alentejo Coast), and Jbel Bou Hachem (Western Rif Range), as we can observe in figure 1. In the southwest of the Iberian Peninsula, the lowland specimens that we sampled, mainly from the Setúbal Peninsula, Sado Estuary and Alentejo Coast (Portugal), Doñana (Spain), and, over the Strait, in Larache and the Rif (Morocco), exhibited morphological characteristics that enabled the identification as *G. ancistrocarpa*.

The specimens that we have observed at two sites on the Western Rif Mountains in Morocco (in Bou Hassim region, above Boubiyene, 1019 m, and above El Maozquir, 1200 m) clearly showed all trifoliate leaves, hence being, after the Iberian Flora key, also *G. ancistrocarpa*. According to many authors, the specimens from those regions were identified as *Genista ancistrocarpa* (Azzoui *et al.* 2000; Benito *et al.* 2006; Romo, 2009; Chambouleyron, 2012) or as the synonym *Genista anglica* subsp. *ancistrocarpa* Maire (Deil *et al.* 2010); such information is available in the Anthos online database also (Anthos, 2013). In both cases, the sampled specimens occurred on tiny swampy depressions with a confined low-rate runoff of groundwater. The surface water had only a few inches depth in the Summer, while it oscillates significantly during the year due to the Mediterranean climate, under which an aestival absence of rainfall prevails. In the Winter, we have observed a small rise, but the runoff was slow as well.

Using the ecological distribution model of these two species in the Iberian Peninsula, the rainfall and height that characterize both sites in the Rif Mountains would predict them to be included within the potential range of *G. anglica*, not the 'lowland' *G. ancistrocarpa*. However, the morphological and genetic data indicate that the sampled plants were unmistakably *G. ancistrocarpa*, despite the elevation, increasing thereby substantially the

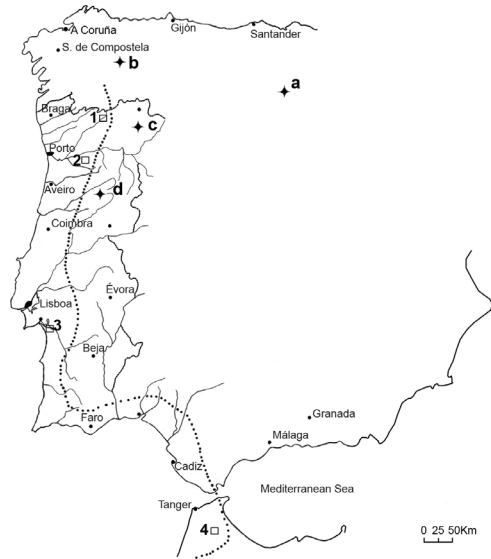


Figure 1. Location of the sampled populations of *G. ancistrocarpa* and *G. anglica*.

Genista ancistrocarpa: 1- Veiga de Chaves - 41°45'29,24"N; 7°27'13,34"W; 350 m. 2- Serra de Montemuro - 40°58'13,74"N; 7°59'20,84"W; 1352m. 3- Sado Estuary - 38°23'04,91"N; 8°36'04,27"W; 25 m. 4- Western Rif (Jbel Bou Hachem) - 36°15'33,45"N; 5°26'30,50"W; 1019m. *Genista anglica*: a- Sierra de Cebollera - 42°07'00,63"N; 2°34'09,63"W; 1100m. b- Sierra de Los Ancares - 42°51'38,20"N; 6°51'20,70"W; 1100m. c- Morais Massif - 41°31'33,52"N; 6°48'17,34"W; 650 m. d- Serra da Estrela - 40°21'33,62"N; 7°39'09,06"W; 1584 m.

height where the latter can occur. The Western Rif Mountains represent the areas with the highest rainfall records in Morocco, with values always over 600 mm, reaching even 2100 mm in some of its highest summits (Rouai & Jaaidi, 2003). The location of the Rif Mountains, from where the specimens from *G. ancistrocarpa* were collected (available on the herbarium João Carvalho & Vasconcellos – code LISI), have the highest precipitation values in the whole Rif Chain and hence across the northwest of Morocco. This climatic originality individualizes the Western Rif as one of the 20 natural areas in which the North of Morocco is divided (Valdés, 2006; Molina-Venegas *et al.*

2013). It is also the natural area with the highest floristic diversity, with an impressive total of 1758 taxa. The occurrence of *G. ancistrocarpa* at high rainy areas, such as the Western Rif Range (NW Morocco), has a parallel case in the Serra de Montemuro (NW Portugal). This fact demonstrates that the range of this taxon must be reviewed. This new mountain occurrences strongly suggest that the range of this taxon, although being chiefly present in lowlands, may also be found in mountain areas, antithetical to the Iberian Flora's reference, which limits its range wholly between 0 and 20 m (Talavera, 1999). On the other hand, it shows that the territorial separation between the two sister species (*G. ancistrocarpa* and *G. anglica*) is essentially biogeographical, based upon isolation of the western populations (*G. ancistrocarpa*), rather than in a clear differentiation of ecological variable gradients (like the height or the rainfall).

MATERIAL AND METHODS

The fresh leaves were collected and stored in a silica gel filled recipient. A first genetic approach was made through the nuclear ribosomal DNA, namely the genes 18S, 5.8S e 28S, and two internal transcribed spacers (ITS1 and ITS2). Four populations of *G. anglica* and *G. ancistrocarpa* were analysed and two plants of each population were sequenced. The PCR protocol used for nrDNA amplification has included an initial denaturation period of 2 minutes at 94°C, followed by 35 one-minute cycles for denaturation at 94°C, for the pairing of the primers with the DNA mold at 54°C, 50s of elongation at 72°C and a single final extension period at 72°C (7 min.). The FITSgenis primers (TCG AAG CCT CACAAG CAG TG) and RITSgenis (CTG AGG TTC CCG TCT TAG G) were especially designed for this work. The sequences were edited with the BioEdit software, version 7.0.1 (Hall,

1999), and also the Clustal X software, version 1.81 (Thompson *et al.* 1997). For the initial approach to the phylogenetic analysis, we used the Bayesian inference through the Mr. Bayes 3.1 software (Ronquist & Huelsenbeck, 2003), using MCMC, with two independent runs of four coupled Metropolis chains of two million generations each, to estimate the distribution of the posterior probabilities. The topologies were sampled every 100 generations and it was estimated a majority consensus tree, after discarding the first 2000 sampled generations.

RESULTS AND DISCUSSION

In figure 2, the resulting phylogram based on Bayesian inference is presented. The analysis of figure 2 shows that the *G. anglica* and *G. ancistrocarpa* constitute two distinct clades, well supported by posterior probability values, confirming the validity of these taxa as distinct entities.

We could not find the specimens of *G. ancistrocarpa* identified in Maâmora region (near Kenitra, after Abderrahman *et al.* 2005), with a similar ecology to the Sado and Doñana regions, but we did find it near Larache. These sublittoral populations were always present in marshy fen complexes along water-logged dune slacks. However, the genetic proximity of the analysed specimens, combined with the preliminary results from the analysis of the samples from Larache and Doñana, confirm the identity between the Western and Southwestern margin of the Iberian Peninsula and Northwestern Morocco, encompassing both coastal populations and the western mountain populations of this taxon.

The low-lying peaty environments along the shoreline of the Iberian-Moroccan Gulf are always associated with the presence of Quaternary sandy deposits, of fluvial or aeolian geomorphogenesis, among dunes (and

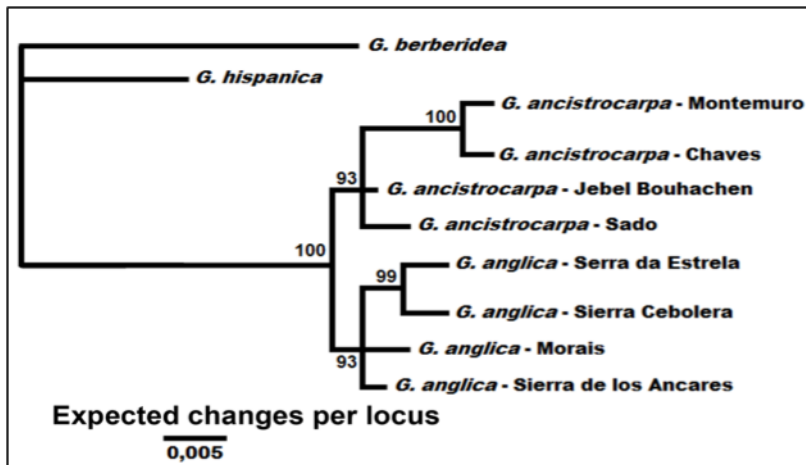


Figure 2. Phylogram based on Bayesian inference of nuclear ribosomal DNA. The Bayesian posterior probabilities are indicated near the nodes.

palaeodunes) or sandy horizontal layers, that induce an exceptional radial hydrology, prone to sustain permanently water-logged surfaces all year round, even during the mediterranean dry summer (Costa *et al.* 1998). So is it in the Sado and the Guadalquivir estuaries, and in the Moroccan fen complexes of Larache and Kenitra as well.

It appears that the *Genista ancistrocarpa* is associated with the presence of these swampy depressions in sandy environments or gravel beds where water infiltrates, constituting extensive subsurface groundwater tables, that permanently provide outlets that feed swampy areas, hence azonal peaty ecosystems. This swampy coastal slacks seem to have provided a coastal migration route for atlantic species during moister phases along the even-numbered MIS of the Pliocene and Pleistocene, and, more recently, during the 8.2 kiloyear event (Costa *et al.* 1998, Honrado *et al.* 2007).

From the North of Aveiro to Doñana ('Coastal Lusitanian-Andalusian' province), the fens on dune slacks or sandy deposits were very common until the beginning of the last century. Due to human action, many swampy

areas in lowlands have been drained for both agricultural use and malaria eradication, or have otherwise become eutrophic.

In Morocco there is another coastal corridor, the so-called 'Atlantic' phytogeographical province, where the sandy areas roughly stretch from Tangier to Rabat (Quezel *et al.* 1988; Galán de Mera *et al.* 2003) with similar traits to those of the west coast sandy corridors of Iberian Peninsula. Likewise does the *G. ancistrocarpa* occur here, always on fens. This is the reason why this taxon was found in marshy areas near Larache (*Genista anglica* L. subsp. *ancistrocapa* (Spach) Maire, after Raynaud, 1979; Quezel *et al.* 1988 and Deil *et al.* 2010, or *Genista ancistrocarpa* after Azzioui *et al.* 2000) and near Mâamora cork-oak forest (Abderrahman *et al.* 2005).

So, this sandy Iberian-Moroccan coastal corridor with swampy fens on the slacks paved the road for southbound migrating Atlantic and Eurosiberian floristic elements towards North Africa (Sauvage, 1961; Dahlgren & Lassen, 1972; Neto *et al.* 2007; Neto *et al.* 2009; Deil *et al.* 2010). Other genetic analyses carried out in the populations of the Iberian coastal corridor

(the ‘Beirense Litoral’ District, the ‘Estremenho Litoral’ District, the ‘Coastal Lusitanian-Andalusian’ Province (Rivas-Martínez, 2007) and west Morocco (Loukkos, Gharb, Atlantic Coast and Mâamora Forest), (Galán de Mera *et al.* 2003, Valdés, 2006) show a clear identity, which demonstrates the importance of this geographical territory in the migration of wetland taxa.

The two clades identified in this study (corresponding to *G. anglica* and *G. ancistrocarpa*) also demonstrate the importance of the Western Iberian Façade as a refuge for mesophilous and hygrophilous species with a strong preference for habitats with edaphic or atmospheric moisture compensation. This moisture becomes available to many species both from the high frequency of summer fogs and the important aquifers close to the surface in the extensive sandy accumulations in the central coast of the Beira Litoral, the Sado Estuary Region and Alentejo Coast in Portugal (Neto *et al.* 2007, Neto *et al.* 2009, Arsénio *et al.* 2009), the Doñana aeolian mantles in Spain (López-Albacete, 2009, Instituto Geológico y Minero de España, 2014) and TangierLarache–Rabat coastal strip in Morocco (Medina *et al.* 2011). These extensive sand accumulations over an conglomerate Pliocene (or earlier) bedrock layers with some impermeability, or smaller sandy deposits over older metamorphic layers (see figure 3), enable the existence of important water supplies that appear in the surface in some lower topographic slacks and thereby explain the existence of sublittoral fens characterized by the presence of: *Erica ciliaris* Loeffl. ex L., *E. erigena* R. Ross, *E. lusitanica* Rudolphi, *Genista ancistrocarpa*, *Carex demissa* Hornem., *Pinguicula lusitanica* L., *Ulex minor* Roth var. *lusitanicus* (Webb) C. Vicioso, *Gentiana pneumonanthe* L. var. *major* (L.) Coutinho, *Myrica gale* L., *Euphorbia uliginosa* Welw. ex Boiss., *Drosera intermedia* Hayne, *Anagallis tenella* (L.) L. *Hypericum elodes* L., *Sphagnum auriculatum*

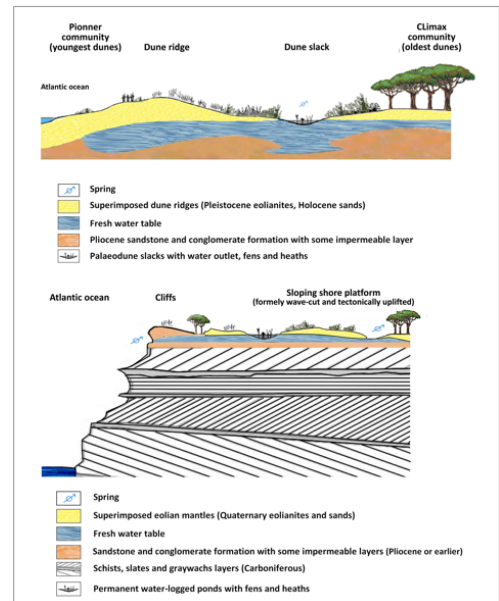


Figure 3. Synoptic theoretical cross-sections on dunes and on coastal flatbeds of SW Iberian Peninsula and NW Morocco.

Schimp., *Thelypteris palustris* Schott, among others (Neto *et al.* 2009). The distribution of the coastal clade of *G. ancistrocarpa* and the used molecular markers led us to the hypothesis of an ancient genetic pattern similar to that found by Pardo *et al.* 2008, for the *Stauracanthus* genus, in the Iberian Peninsula. In that work, the authors argue that the hot and dry conditions, which prevailed during the Late-Miocene Messinian Salinity Crisis (Hsü *et al.* 1977), might have driven the populations of *Stauracanthus spectabilis* Webb to retreat into isolated refuges in the westernmost sectors on both sides of Gibraltar Strait (SW Portugal and Spain versus NW Morocco), where milder climatic conditions still stood under the influence of the Atlantic Ocean.

The legacy of the Late Miocene and Pliocene climate changes on the genetic patterns of many animals and plants in the Iberian Peninsula seems therefore to gain a

quintessential importance above the genetic signal of the Quaternary cold phases and temperature decrease long-term trend, after phylogeography works by many authors.

Paulo *et al.* 2001 have studied the phylogeography of the Iberian Lizard (*Lacerta schreiberi* Bedriaga) and suggest that earlier events in the Pliocene might have triggered the main divergence between populations. The setting of the Mediterranean climate with a dry hot season has been the first important event that has separated several populations that, in their turn, may have evolved further apart during the Pleistocene glaciations. The study of genetic variation and relationships among *Ulex* species in southern Spain and Northern Morocco strongly supports the importance of Mid-to Late Miocene events, primarily associated with the reopening of the Mediterranean Sea in Gibraltar (Cubas, 2005). So shows the pattern of genetic variation in the golden-striped salamander, *Chioglossa lusitanica* Bocage, based on the work by Alexandrino *et al.* 2000. These authors suggest that the former isolation of at least two population units in the southern part of the present-day species distribution would correspond with a genetic isolation of 1.5–3 MY, i.e., from the late Pliocene/early Pleistocene onwards. This matches the hypothesis that the first important glaciation in the Pliocene (as it corresponds to a climatic shift towards greater dryness and cold) has only played an a posteriori role on the genetic patterns of Iberian populations of many animal and plants, frequently less visible than the genetic imprint left by the paramount biogeographic event which was the opening of the Strait of Gibraltar. Henceforth the subsequent genetic separation of most species with a North African and Iberian distribution (Schmitt, 2007; Habel *et al.* 2009) was present already when the Pleistocene glaciations arrived, conclusion common to a large number of papers published in recent years for amphibians (Plötner, 1998; García-París & Jockusch, 1999; Steinfartz *et al.*,

2000; García-París *et al.* 2003; Carranza *et al.* 2004; Fromhage *et al.* 2004; Martínez-Solano *et al.* 2004; Martínez-Solano, 2004; Veith *et al.* 2004), shrews (Cosson *et al.* 2005), scorpions (Gantenbein & Largiadèr, 2003) and plants like the *Stauracanthus* genus, (Pardo *et al.* 2008), *Carex helodes* Link (Escudero *et al.* 2008), *Bellis annua* L. (Fiz *et al.* 2002), *Quercus suber* L. (Ajbilou *et al.* 2006), *Q. ilex* L. (Lumaret *et al.* 2002), *Pistacia lentiscus* L. (Werner *et al.* 2013, Valdés, 2006; Lavergne *et al.* 2013).

CONCLUSIONS

The *G. ancistrocarpa* coastal distribution seems to correspond more with an ancient genetic pattern rather than a recent separation event, which might have derived from the Late Miocene tectonic and climate changes. The transition from a moist and warm climate to a dry one, which marks the Messinian Salinity Crisis at the Miocene–Pliocene boundary 5.96–5.33 MY (Hsü *et al.* 1977; Krijgsman *et al.* 1999; Loget & van den Driessche, 2006), is likely to have thrust a pressure upon a part of the ancient wet-soil *Genista* populations, just like the one which has originated the genetic differentiation of *Stauracanthus spectabilis* subspecies (Pardo *et al.* 2008). Another work that we are undertaking on *Gentiana pneumonanthe*, points towards the same genetic isolation of western populations apart from the central mountain chains in the Iberian Peninsula. The establishment of a Mediterranean climate type at Late Pliocene 3.2–2.8 MY (Suc, 1984), as well as the Pleistocene glacials, might have cohered this already present western isolation pattern. More recently, the anthropic pressure, with its consequent habitat decrease and fragmentation, magnified the conditions prone to the internal genetic differentiation of this western coastal clade, evident in subpopulations of the infraspecific taxa of *Stauracanthus spectabilis* (subsp. *spectabilis* e

subsp. *vicentinus* (Daveau ex Cout.) T.E. Díaz, Rivas Mart. & Fern. Gonz.) in SW Portugal. The same phenomenon might have driven the genetic differentiation between the portuguese, spanish and moroccan *Genista ancistrocarpa* western populations. Notwithstanding, further research is needed both with field and lab work to reinforce the first results. Regarding the *G. anglica* clade, it may correspond to the ancient populations of wet-soil *Genista*, which have been held further inland in the Iberian Peninsula during Quaternary rextasy phases, possibly sheltered in multiple refugia that new works may be able to pinpoint, but whose evolution can be similar to the process described by Paulo *et al.* 2001 for the Iberian Lizard and by Font *et al.* 2009 for the *Centaurea* Genus (*Acrocentron* section). Apparently some populations could remain in multiple inland refugia, assumably by colonizing mountains during warm phases and moving downward into milder valleys during cold phases, tracking convenient biotopes since the Late Pliocene period. This fact underlines the importance of this geographical hilly Iberian-Moroccan territory, which provides multiple niches for the refuge and migration of taxa associated with wetlands inside the Peninsula and NW Morocco, hence focal points for research and conservation.

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